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Early IL-2/sIL-2R surge following surgery leads to temporary immune refractoriness

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SUMMARY

High serum level of immunoreactive but not biologically active IL-2 was detected 1 day after surgery in patients undergoing major operation (abdominal, open-heart), in proportion to the tissue injury caused by surgical trauma. IL-2 values were highest in those patients who underwent open-heart surgery and received blood transfusions. In all patients they declined in the third and fourth post-operative days. Elevated serum levels of soluble IL-2 receptors (sIL-2R) were already present 1 day after operation, and peaked in the third and fifth post-operative days after mitogen triggering. Blood lymphocytes derived from operated patients secreted reduced amounts of both IL-2 and sIL-2R compared with control lymphocytes. The extent and duration of this reduction were also proportional to the tissue trauma and were affected by blood transfusions. Based on these data we suggest that early post-operative systemic immunological activation (appearance of IL-2 in the serum) is followed by elevation of sIL-2R, which then interferes with IL-2-dependent immunity. Blood lymphocytes are probably not involved in the post-operative immunological activation. The trigger for and the site of IL-2/sIL-2R synthesis are not yet clear.

Keywords IL-2 soluble IL-2 receptor open-heart surgery

INTRODUCTION

Major operations, especially cardiac surgery, can cause changes in cell-mediated immunity [1]. The decline in peripheral lymphocyte function, which lasts 3–7 days after surgery, was attributed to intrinsic lymphocyte changes or to redistribution of reactive T cells from blood to tissues [2] and to inhibitory effects of serum factors like prostaglandins and corticosteroids [3–6].

The T cell activation process is associated with generation of IL-2 and surface expression of its receptors (IL-2R). The IL-2R can detach from the cell surface after *in vitro* stimulation and be detected in soluble form in the surrounding milieu [7]. Elevated soluble IL-2R (sIL-2R) concentrations have been found in AIDS [8], during the active phases of certain autoimmune diseases [9–11], organ transplant recipients where the level of sIL-2R was correlated with the frequency and intensity of rejection [12,13], in patients with lymphomas and leukaemias [14], and after burns [15]. sIL-2R is a natural blocker of IL-2 [16], but its biological importance is controversial.

The results of this study point to a causual relationship between post-operative serum levels of IL-2 and sIL-2R, and suggest that sIL-2R could be considered as a 'serum factor' responsible for post-operative immune deficiency.

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PATIENTS AND METHODS

Patients

Twenty male patients, aged 40–65 years, underwent coronary artery bypass (CABG), cardioplegia and moderate core hypothermia (24–30°C). Anginal syndrome without congestive heart failure was the indication for surgery. In all, no patient in this series had a history of malignancy or verified immunological disorder. All patients were classified in the New York Heart Association (NYHA) classes II and III. Ten of them received blood transfusions from random donors (CABG A) and the other 10 did not (CABG B). All 20 patients had uncomplicated recovery without evident inflammatory processes after the operation. Ten patients undergoing uncomplicated cholecystectomy without transfusion and 15 healthy volunteers in the same age range were examined as a control group. Samples of serum were taken before the operation, 24 h, 72 h and 144 h after the end of surgery, and kept at −70°C until assay. Heparinized blood was also collected and immediately subjected to analysis of T cell subsets and T cell IL-2 and sIL-2R secretion. Determination of sIL-2R and IL-2 levels was performed in all patients and controls simultaneously.

Assessment of sIL-2R and IL-2

Quantification of sIL-2R and IL-2 levels in sera or supernatants was made using an ELISA based on MoAbs to two different

epitopes of the Tac molecule (T Cell Sciences Inc., Cambridge, MA) and Intertest 2 (Genzyme, Boston, MA), respectively, according to the manufacturer's specifications. IL-2 and sIL-2R in supernatants were measured after T cell culture of 24 h and 120 h, respectively, since these culture periods were found to be optimal for IL-2 and sIL-2R measurements [17]. Biological activity of IL-2 was quantified using the cytotoxic T cell line CTCL, which was maintained in RPMI 1640 with 10% fetal calf serum (FCS) and IL-2 at 1000 U/ml. Cytotoxic T cell line cells were washed and adjusted to a concentration of 10^5/ml with complete medium. Cells were cultured in 100 μl of IL-2 containing supernatants or supernatants diluted with complete medium. After 22 h, ^{3}H-thymidine was added, and at 26 h the cells were harvested and counted in a liquid scintillation counter. All cultures were performed in triplicate. Results were compared with a standard curve generated by adding known amounts of recombinant IL-2 (Sigma) to CTCL.

Analysis of lymphocyte subsets

Peripheral blood mononuclear cells (PBMC) were tested for total T, CD4$^+$ and CD5$^+$ cells using MoAbs obtained from Becton Dickinson (Mountain View, CA). We used the indirect fluorescence method and the positive cells were enumerated by FACS.

Culture conditions for IL-2 and sIL-2R production

T cells were isolated from PBMC using the rosette technique and were activated with phytohaemagglutinin-P (PHA-P), 0·5 μg/ml, for 24 h and 120 h. The cells were incubated in flat-bottomed 96-well plates in RPMI 1640 ± 10% FCS + 1% glutamine + 1% antibiotics (penicillin, streptomycin). Cells were cultured at 10^6 cells/ml, 0·2 ml/well. Supernatants were collected and kept at −70°C until assayed.

RESULTS

Serum concentration of IL-2 and sIL-2R (Figs 1 and 2)

Preoperative serum levels of sIL-2R in all four groups (CABG A + B, cholecystectomy and controls) did not differ (Fig 1). Twenty-four hours (1 day) after surgery a significant elevation ($P < 0·01$ or $P < 0·05$) of serum sIL-2R was measured in CABG patients. Seventy-two hours (3 days) after surgery the highest value of sIL-2R was measured in sera derived from both CABG and cholecystectomy patients, and was significantly higher than that of controls. The mean sIL-2R value in the sera of transfused CABG patients was higher ($P < 0·05$) than that of non-transfused CABG patients and of patients undergoing cholecystectomy. Seven days following surgery, sIL-2R levels in CABG B and cholecystectomy patients returned to preoperative values, but that of CABG A patients, although declining, was still significantly higher ($P < 0·01$) (Fig. 1). No immunoreactive IL-2 could be measured in the serum of normal controls and of preoperative patients. One day after the operation immunoreactive IL-2 was detected in serum derived from most operated patients. The serum value of IL-2 in CABG A patients was significantly higher than that of the two other groups. Three days after surgery IL-2 levels declined, and IL-2 concentration of CABG A was still the highest. After 7 days no IL-2 could be detected in the serum of CABG B and cholecystectomy patients, but the serum of most CABG A patients still contained immunoreactive IL-2 (Fig. 1). In view of the parallel trends in the serum content of both immunoreactive IL-2 and sIL-2R, the

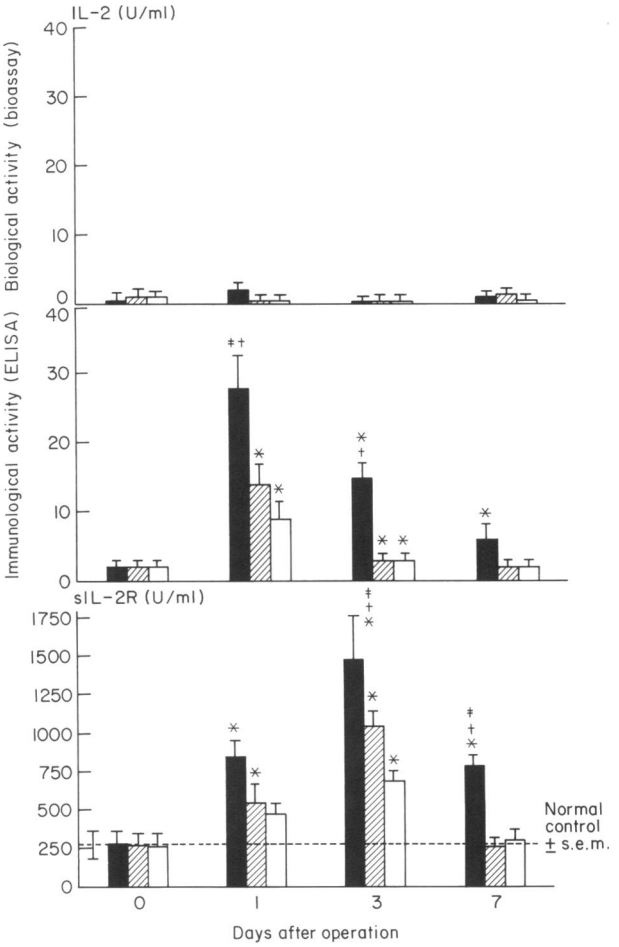

Fig. 1. Serum concentration of IL-2 and soluble IL-2 receptors (sIL-2R). Significant immunoreactive (but not bioactive) IL-2 was observed 1 day after the operation and declined later. Serum sIL-2R peaked in the third post-operative day. Both mean IL-2 and sIL-2R values were highest in coronary artery bypass (CABG) A, while CABG B patients had higher values than cholecystectomy (Chol.) patients. * Significantly higher than control ($P < 0·05$); † significantly higher than Chol. ($P < 0·05$); ‡ significantly higher than CABG B ($P < 0·05$). ■, CABG A; ▨, CABG B; □, Chol.

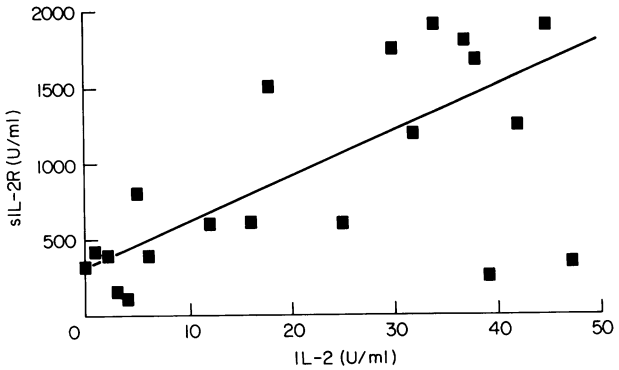

Fig. 2. Levels of immunoreactive serum IL-2 and soluble IL-2 receptors (sIL-2R) in individual operated patients 1 (IL-2) and 3 (sIL-2R) days after surgery. The correlation coefficient (r) was 0·58 and the significance $P < 0·05$.

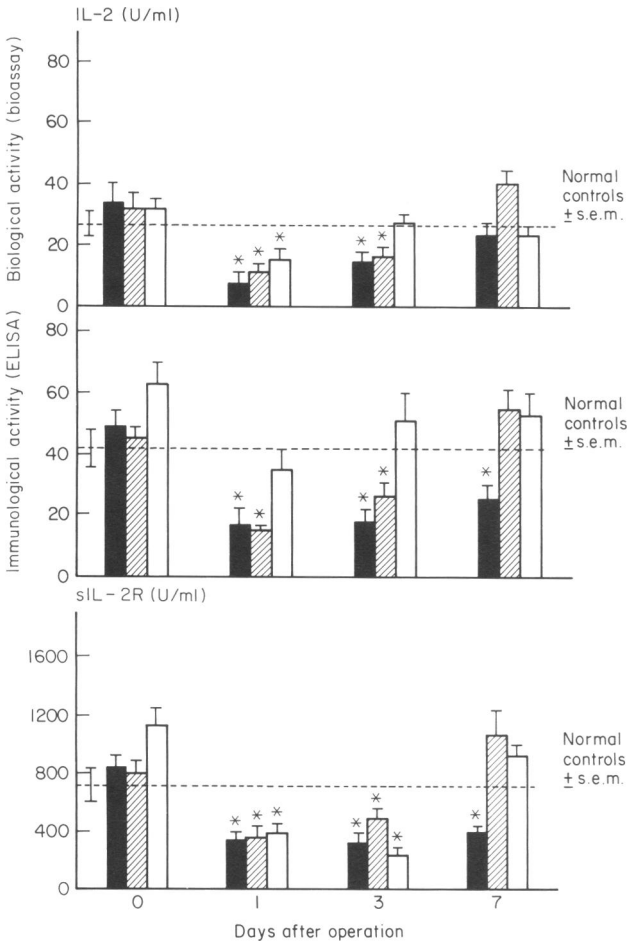

Fig. 3. Secretion of IL-2 and soluble IL-2 receptors (sIL-2R) by *in vitro* stimulated peripheral lymphocytes. All operated patients showed reduced ability to secrete IL-2 and sIL-2R 1 day after surgery. Only in coronary artery bypass (CABG) A patients was this reduction still observed on the seventh post-operative day. In contrast to serum, secreted immunological and biological IL-2 activities parallel. * Significantly less than control values ($P<0.05$). ■, CABG A; ▨, CABG B; □, Cholecystectomy.

relationship between immunoreactive IL-2 levels to its soluble receptor was evaluated by linear regression analysis (Fig. 2). sIL-2R values from 20 patients in the third post-operative day were correlated with their first day immunoreactive IL-2 levels. A significant correlation ($r=0.58$, $P<0.05$) was found. Practically, no biologically active IL-2 could be detected in sera derived before or after surgery.

IL-2 and sIL-2R secretion by T cells (Fig. 3)

T cells derived from operated patients 24 h after the operation demonstrated a significant suppression in their ability to secrete bioactive and immunoreactive IL-2 after PHA stimulation ($P<0.05$), which was more pronounced in CABG patients. After 3 days the IL-2 levels produced by cholecystectomy patients were normalized. Seven days after the operation secreted IL-2 levels of CABG B patients returned to preoperative normal values, while those (immunoreactive only) of CABG A were still lower ($P<0.05$). sIL-2R secretion by activated T cells, which did not differ from normal secretion before surgery,

Table 1. T cells and T cell subpopulation after surgery

	0	1 day	3 days	7 days
N	64·3 ± 18·5	—	—	—
CABG A	60·7 ± 15·9	44·7 ± 15·3*	53·1 ± 12·8	63·9 ± 15·4
CD3 CABG B	61·9 ± 14·6	46·1 ± 12·4*	51·8 ± 14·7	64·5 ± 10·6
Chol.	71·5 ± 15·8	54·3 ± 12·7*	66·9 ± 12·3	73·4 ± 14·4
N	39·8 ± 12·4	—	—	—
CABG A	44·7 ± 15·2	27·1 ± 17·3*	31·2 ± 10·9	40·8 ± 14·2
CD4 CABG B	41·3 ± 12·9	25·7 ± 8·2*	37·8 ± 15·4	42·1 ± 11·6
Chol.	43·2 ± 14·6	33·2 ± 14·8*	35·4 ± 11·7	38·6 ± 12·6
N	22·0 ± 10·9			
CABG A	24·0 ± 12·3	20·6 ± 5·2	17·5 ± 6·9	22·6 ± 6·0
CD8 CABG B	22·7 ± 9·8	16·7 ± 6·3	20·2 ± 3·6	21·5 ± 4·4
Chol.	23·5 ± 11·2	25·3 ± 10·2	24·2 ± 11·7	24·6 ± 10·3

0, Preoperation; Chol., cholecystectomy.

Total T-CD3. Helper/inducer T: CD4. Suppressor/cytotoxic T: CD8.

* Significantly reduced compared with preoperative levels.

was reduced significantly in all operated patients 1 and 3 days after the operation, and returned to normal values on the seventh day in non-transfused CABG patients and in cholecystectomy patients. They were still low ($P<0.05$) in transfused CABG A patients.

T cell subpopulations (Table 1)

Normal relative and absolute CD3, CD4 and CD5 T cell numbers were present before operation in the peripheral blood of patients undergoing CABG A, CABG B and cholecystectomy. One day after operation reduced CD3 proportions ($P<0.05$) were detected in most patients, and normalized on the third day. Reduced CD4 percentage on the first post-operative day was also resolved after 3 days. CD5 T cell percentage was unchanged after surgery (Table 1). Significantly reduced CD4/CD5 ratio was observed in CABG A and cholecystectomy patients 1 day after surgery, and normalized towards the third post-operative day.

DISCUSSION

In this study we have found that serum levels of both immunoreactive IL-2 and sIL-2R rose after surgery. In contrast, peripheral T cells activated by PHA had diminished ability to secrete IL-2 and sIL-2R for at least 3 days after surgery. The highest serum levels of IL-2/sIL-2R and the most prolonged inhibition of T cell activation were observed in transfused CABG A patients.

In equilibrium, a low basal secretion of IL-2 and its rapid metabolism result in undetectable IL-2 levels. Low IL-2 concentration maintains steady levels of membrane and secreted IL-2 receptors [18], thus controlling systemic immune activation. High serum sIL-2R may reflect, therefore, prior *in vivo* systemic activation of the immune system and the presence of high levels of endogenous IL-2. This assumption is supported by the appearance of IL-2 transcripts before those of IL-2 receptors after *in vitro* activation of T cells [19], and by the early detection of IL-2 and late detection of sIL-2R in culture supernatants

derived from triggered T cells [17]. In our study, accordingly, mean peak levels of systemic IL-2 in operated patients preceded those of serum sIL-2R. Moreover, peak levels of individual sIL-2R were significantly correlated with those of IL-2. Thus, a causative role of IL-2 in sIL-2R secretion after surgery is suggested.

In spite of the high immunoreactive serum IL-2 levels, no consistent bioactive serum IL-2 could be detected. It has previously been shown that sera from traumatized donors are non-specifically toxic to T cell lines, some of which are used in the bioassays [20]. Also, sIL-2R has been found to bind IL-2, prevent its binding to membranal receptors and inhibit IL-2-dependent T cell proliferation, on which bioassays are based [17]. Twenty-four hours after surgery, serum sIL-2R levels were already high, and thus able to interfere with bioactive but not immunoreactive IL-2. More importantly, if *in vivo* IL-2 secreted early after surgery is indeed bound by serum sIL-2R, the high systemic immunoreactive IL-2 concentrations are not functional. Elevated long-lasting free sIL-2R, first triggered by IL-2, could thus be added to the list of serum inhibitory factors which temporarily prevent immune activation after major surgery. Indeed, in patients with visceral leishmaniasis [21,22], after thermal injury [23] or receiving adoptive immunotherapy with rIL-2 and lymphokine-activated killer (LAK) cells [24], high serum levels of sIL-2R have been found and were shown to inhibit IL-2 generation and IL-2-dependent responses.

Immunological functions have been found to be more depressed after cardiac operations than after other forms of surgery [25]. CABG patients undergoing blood transfusions have been shown to be more immunocompromised and for longer periods than non-transfused CABG patients [1]. More severe stress, tissue and bone damage characterize open-heart surgeries, in addition to the use of cardiopulmonary bypass machine and hypothermia, which may be traumatic. As to transfused CABG patients, it has been suggested that the amount of blood loss *per se* or activation of non-specific suppressor lymphocytes by foreign blood cells are responsible for their more accentuated abnormal immunity [26–28]. It has been shown that allogeneic cells produce rapid accumulation of sIL-2R [29], thus explaining the high values of the soluble receptors in the transfused patients. The mean serum IL-2 and subsequent sIL-2R levels, which were higher after CABG than after cholecystectomy and highest in CABG patients undergoing transfusion, could therefore be linked to reduced immunity.

Twenty-four hours post-operatively, a reduced proportion (and absolute number (data not shown)) of blood CD3 and CD4 T cells was found, which normalized after 3 days. Depletion of $CD4^+$ T cells, which are the main producers of IL-2, could account for the early reduced peripheral responsiveness to PHA. It has recently been suggested that the immunological changes observed in the early post-traumatic stage (initial 3 days) are due to such CD4 T cell redistribution from the peripheral blood to the tissues [2]. Alternatively, peripheral T cells could be affected by the early high systemic IL-2 levels and become refractory to further stimulation by mitogen. The reduced capacity of IL-2-stimulated T cells for further activation through the T cell receptor has been described [30].

A mechanism involving early high systemic levels of IL-2 and subsequent sIL-2R could play a role in post-operative immune deficiency. However, we do not yet know the location of IL-2 and sIL-2R synthesis, and what is the trigger for the early post-operative IL-2 secretion. We have chosen only patients with uncomplicated recovery. The *in vitro* immunologic refractoriness of most of them was resolved during and towards the end of the first post-operative week. To decide whether high serum levels of IL-2 or sIL-2R could predict post-operative infections, more patients with heterogeneous post-operative behaviour have to be studied.

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